

## 19. Cooldown to Cryogenic Temperature

After cryoprotective perfusion, a patient must be cooled to a temperature that is low enough to inhibit all potentially harmful chemical reactions, including those caused by the toxicity of any cryoprotectants that have been introduced.

In 1889, the Swedish physicist and physical chemist Svante Arrhenius proposed a formula to derive the rate of a chemical reaction from the temperature at which it occurs, in degrees Kelvin, using various constants specific to the chemicals involved. This formula is now known as the Arrhenius Equation. For many common chemical reactions occurring in mammalian biology, the equation predicts that the reaction rate will be approximately halved for each decrease of temperature of 10 degrees Celsius. This is known as the “Q10 rule,” which we have mentioned in Section 11. The rule applies approximately down to 0 degrees C, when water freezes in human tissue.

If the water in tissue is replaced with appropriate cryoprotectant chemicals, they may remain liquid at temperatures below -100 degrees C. Tissue containing lower concentrations of cryoprotectants may still freeze, but areas of frozen tissue between ice crystals will remain liquid.

Chemical reactions in the high sub-zero range (tens of degrees below 0 degrees C) are dominated by cryoprotectant toxicity. This is a poorly understood side effect of cryoprotectants, causing cells to lose viability, meaning that their ability to resume functioning spontaneously after rewarming will be impaired.

Injury from toxicity occurs on a timescale of minutes when cells are exposed to high concentrations of cryoprotectants, but the rate of damage decreases with temperature. Recognizing this relationship, we apply a relatively low concentration of cryoprotectant as cooling begins, and increase the concentration as the temperature drops, reaching a terminal concentration around -80 degrees C.

Cryoprotectant solutions become extremely viscous, like thick syrup, below  $-50$  degrees C. The high viscosity becomes a more important factor than the Arrhenius equation in slowing chemical reactions. As viscosity increases exponentially according to a relation called the Vogel-Fulcher Equation, chemical reactions become inhibited by the inability of molecules to move by diffusion to reach each other. At a temperature of  $-110$  degrees C, a molecule in a cryoprotectant of the type used by Alcor would need 100 years to travel as far as in one minute at 0 degrees C.

At temperatures below approximately  $-125$  degrees C, cryoprotectant viscosity becomes so high (10 trillion Poise) that cryopreserved tissue becomes glasslike and behaves as a solid. At this point the tissue is said to be vitrified, and its transition from liquid to solid is known as vitrification. The temperature at which this occurs is known as the glass transition point, often denoted as  $T_g$ . Molecules become immobilized, and chemical changes become impossible.

However, an undesirable process known as ice nucleation can still occur. Vitrification solutions used in cryonics are metastable, meaning that below a certain temperature ( $-5$  degrees C for the solution known as M22) growth of ice is thermodynamically favored. We can minimize this risk by cooling as quickly as possible, so that ice doesn't have time to grow even though the cold liquid "wants" to form ice.

At very low temperatures, the thermodynamic tendency to form ice is so great that water molecules will rotate locally to join with their neighbors to create extremely tiny crystals known as ice nuclei. Lateral motion by diffusion is not necessary for this to occur. Therefore ice nucleation can happen even near  $T_g$  and a few degrees below it, until the process finally ceases.

Ice nuclei are tiny and harmless nanometer-sized objects, but are undesirable because when tissue is warmed in the future, the nuclei will have an opportunity to grow into ice crystals that are large enough to cause injury. For this reason, tissue is preferably stored at temperatures far enough below  $T_g$  to inhibit ice nucleation. The time and temperature dependence of ice nucleation, and whether it ever ceases during storage slightly below  $T_g$ , are issues that remain poorly understood.

We know of no published reports of ice nucleation at temperatures below  $-135$  degrees C. Additional cooling below this point is therefore considered unnecessary, but most patients are cryopreserved in liquid nitrogen, which has a boiling point of  $-196$  degrees C. The reasons for using liquid nitrogen, and the possibilities for other modes of storage, are discussed in Section 20.

Regardless of which temperature is used, the patient must be cooled to get there, and this is often referred to as the “cooldown phase” preceding long-term cryopreservation.

## **Cooling Fundamentals**

In Section 11, under the subhead “The Physical of Cooling,” we discussed the concept of heat transfer. Cooling occurs when heat is transferred from a location that is relatively hot to a location that is relatively cold, via convection, conduction, or radiation. Heat transfer will tend to continue until the system equilibrates, meaning that the temperatures become equal.

If all other factors are constant, cooling will be most rapid if the temperature difference between a hot location and a cold location is as great as possible. If the temperature difference diminishes as a result of heat transfer, the rate of cooling will also decrease.

Cooling will also be more rapid if the ratio of the surface area to the mass of the object to be cooled is as great as possible, and if cooling can be applied internally as well as externally. This is why blood is circulated by cardiopulmonary support during initial stabilization of a cryonics patient, and why perfusion cooling by heart-lung bypass is the most rapid method of initial cooling, removing heat from the body internally. For these same reasons, various proposals have been made to cool the body or cephalon of a cryonics patient, after cryoprotection, by perfusion with a substance that remains liquid or gaseous all the way down to storage temperature.

Fluorocarbon compounds which remain liquid at temperatures as low as  $-120$  degrees C, and cold helium gas, have been pumped through blood vessels experimentally. Technical challenges have so far discouraged this approach in cryonics cases, and cryogenic cooling of patients has always been done by removing heat from the outside of the body. This means that heat

must travel outward from the center of the patient to the skin, where it is removed by a chilled gas or liquid.

## **Fracturing**

CT scanning studies have shown that the entire brain of a patient cryopreserved under ideal conditions is capable of vitrification. Tissues elsewhere in the body do not all vitrify, and may form ice to varying extents. Still, the presence of cryoprotectant limits ice formation to much less than would form in absence of cryoprotectant.

Vitrification entails a loss of plasticity in tissues. As they become more brittle, they are less able to tolerate stress caused by unequal thermal contraction between locations of slightly differing temperature. To minimize this risk, the cooling rate is typically paused just above  $T_g$  to allow time for temperature equilibration, which we hope will relax mechanical stress.

Cooling to storage temperature must then proceed slowly, at no more than 1 degree Celsius per hour, as the vitrified patient has become increasingly vulnerable to fracturing caused by thermal stress. Although this stress can be minimized, there is presently no cooling protocol known to avoid all fracturing of human brains or bodies stored at the temperature of liquid nitrogen ( $-196$  degrees C). We believe that some large fractures will always occur. This problem has been discussed in detail in “Systems for Intermediate Temperature Storage for Fracture Reduction and Avoidance,” an article published in *Cryonics* magazine and accessible on the Alcor web site.

The exact cooling profile will be recommended by the laboratory that developed the cryoprotectant, and the profile may be updated from time to time as new data becomes available. Past practice has been to pause cooling at  $-110$  degrees C. More recent practice has favored pausing the rapid phase of cooling at temperatures as warm as  $-80$  degrees C to permit better relaxation of thermomechanical stress before continuing to cool more slowly through the glass transition temperature and below.

### *Alcor's Sonic Fracture Detection System*

To detect possible fracturing events, Hugh Hixon at Alcor developed a “crackphone” that is sensitive to the characteristic sound of mechanical fractures. This device uses a sensing element from an ultrasound scanner, and performs computer processing of the signal.

At the conclusion of cryoprotective perfusion, a crackphone sensor is inserted through each burr hole in the skull so that it rests between the skull and the dura around the brain. Wax is then used to fill the burr holes around the wires from the sensors. Ideally, Hixon has stated that he would prefer to insert each sensor into the brain itself, but feels that this would be unacceptable because of the injury it would cause.

Hixon believes that fracturing occurs primarily because the coefficient of thermal contraction of the brain differs from that of the skull, although other causes may be possible. If his theory is correct, fracturing should be less common in cases where more shrinkage of the brain has occurred, so that it has separated from the skull. As of 2017, no one has reviewed case data to determine if such a relationship exists.

While the patient is cooling, output from each sensor passes through an analog-digital converter to a computer where software recognizes and timestamps each fracturing event. A typical event lasts for about 4 milliseconds.

In 2011, CT scanning of several Alcor patients after cooling to liquid nitrogen temperature revealed that the crackphone sensors that had been placed were not in direct contact with the brain surface. Events detected by the crackphone may not have been fractures, or may have been fractures that occurred elsewhere. Although organs as large as the human brain are known from direct observation to fracture often during cooling to liquid nitrogen temperature, as of 2018 there has been no correlation confirming that events observed by the crackphone are actually fractures in the brain being monitored.

## **Whole-Body Cooldown: History at Alcor**

In the late 1980s, Hixon built a large insulated bath at Alcor that could be filled with silicone oil (see Figure 19-1). A whole-body patient ready for cryogenic cooling was wrapped in plastic and strapped to a wire-mesh stretcher that was lowered into the oil. The bath was then cooled very simply by adding large pieces of dry ice. Cooling was enhanced by using a submersible pump to circulate the oil around the patient.



*Figure 19-1. Hugh Hixon sitting beside the silicone-oil cooldown box that he designed. It is now used for cooling patients in rapidly circulating liquid nitrogen vapor.*

Frozen carbon dioxide does not transition through a liquid phase when it warms. It changes directly into a gas in a process known as sublimation. Thus, when the pieces of dry ice absorbed heat from the silicone oil, they turned into carbon dioxide gas that was vented harmlessly into ambient air.

At the end of a case, Hixon dried the silicone oil (that is, he removed water from it) using a technique that he devised using plaster of paris. Even though the oil was relatively expensive, it could be reused, and the silicone bath was a simple and economical way to achieve whole-body cooling.

It was also quite efficient, as frozen carbon dioxide absorbs 574 kilojoules per kilogram of latent heat when sublimation occurs. No increase in temperature is associated with this change of state, as the heat is used entirely to break molecular bonds that were created when the substance was frozen.

A disadvantage of Hixon's system was that it could not cool the patient below  $-79$  degrees C, the temperature at which dry ice begins to vaporize. Moreover, the cooling rate of the patient diminished asymptotically as body temperature neared the temperature of the oil.

Cooldown therefore required an additional phase, in which the patient was removed from the oil bath, protected from mechanical injury by being enclosed in a sleeping bag, and suspended in a dewar. At fifteen-minute intervals, liquid nitrogen was injected into the dewar where it vaporized, lowering the temperature toward  $-196$  Celsius over approximately one week. A fan circulated the vapor to achieve uniform cooling, and eventually the temperature dropped sufficiently for the nitrogen to remain liquid. As more liquid nitrogen was added, the level rose until the patient was fully immersed. At this point the patient was placed in a pod fabricated from sheet aluminum for transfer to permanent storage in one of Alcor's storage dewars.

## **Whole-Body Cooldown: Current Protocol at Alcor**

Prior to 2005, Alcor used glycerol as a cryoprotectant. This reduced the amount of ice that formed during cooling, but still allowed tissue to freeze significantly. It required slow cooling to allow cells to dehydrate between growing ice crystals, so that they didn't freeze intracellularly. When the M22 vitrification solution was introduced, it was capable of vitrifying without

forming any ice at all but, as already noted, required rapid cooling to  $T_g$  to minimize toxicity.

To achieve rapid cooling, the original silicone oil bath was repurposed to use cold nitrogen gas. The bath is still being used for that purpose as of 2018. As before, the patient is wrapped in plastic sheet and strapped to a wire-mesh stretcher before being lowered into the box. The patient cannot be enclosed in an aluminum pod, because the standard Alcor pod is slightly too long to fit.

A separate lid is placed over the cooling box. The lid contains a plenum and a fan, which circulates vapor actively over the skin of the patient. Liquid nitrogen is injected into the box where it absorbs heat by evaporating, generating cold nitrogen gas. Typically about 100 liters of liquid nitrogen are lost through evaporation during each 24-hour period in which the box is being used.

Alcor uses computer control to maintain a programmed target temperature of cold gas inside the cooling box. If good cryoprotection has been achieved, initial cooling in vapor is as rapid as possible.

The target temperature of gas in the box used to be  $-110$  degrees C, but more recently  $-80$  degrees C has been used for whole body patients to allow better relaxation of themomechanical stress before cooling more slowly to below the glass transition temperature.

If cryoprotective perfusion has been impossible as a result of blood clotting or other circulatory problems, the initial rate of cooling is greatly reduced to allow time for cells to dehydrate by osmosis during freezing of water outside cells. This freeze-induced dehydration of cells, a standard practice in the field of cryobiology during cryopreservation by freezing, prevents water from freezing inside cells (intracellular ice formation) which is more damaging than ice growing in between cells (extracellular ice formation).

When the target temperature is reached, the patient is removed from the cooling box. The second phase of the cooldown is the same as was used formerly. The patient is placed in an opened sleeping bag that has been sprayed with liquid nitrogen. The bag is then closed and is suspended in an A9000 model dewar made by MVE, originally used for long-term storage of



cryopatients but repurposed for cooldown. Cooling then proceeds over a period of days.

Finally the patient is removed for immersion in liquid nitrogen in one of Alcor's storage dewars. The transfer process for a whole-body patient takes about 30 minutes, which Hixon finds unsatisfactory, because some warming must inevitably occur.

## **Whole-Body Cooldown at The Cryonics Institute**

For many years the Cryonics Institute omitted the rapid cooling phase used at Alcor. The patient was placed in a sleeping bag immediately after cryoprotective perfusion and was suspended in a cryostat for cooling by vapor. This procedure was comparable to the second phase of cooldown at Alcor, but was not controlled in any way. The thermal insulation of the sleeping bag, coupled with absence of any method to circulate nitrogen vapor actively, meant that the entire process proceeded very slowly.

This attracted criticism that the slow process allowed far too much opportunity for cryoprotectant toxicity to cause injury. In response to this criticism, when Ben Best became president of the organization he designed an insulated enclosure in which nitrogen liquid was introduced through a tube perforated with nozzles. The liquid vaporized almost instantly as it emerged through the nozzles, and a fan circulated the vapor around the patient. This system provided faster cooling than the one which it superseded, and is still in use. It may be comparable to the whole-body system being used at Alcor. Regardless of any debates that occurred in the late 20th century regarding cooling rates used for frozen patients perfused with glycerol, fast initial cooling after cryoprotective perfusion became mandatory at both CI and Alcor with their switch to vitrification solutions in the first decade of the 21st century.

## **Neuropatient Cooldown at Alcor**

Hugh Hixon used silicone oil experimentally on neuro patients before he built the bath for whole-body patients, but within a few years he started cooling

cephalons in nitrogen vapor from start to finish. This system is still being used at Alcor at the time of writing. One version is shown in Figure 19-2 and Figure 19-3.



*Figure 19-2. A neuro cooldown dewar at Alcor. Frost has accumulated from water vapor in the air while cooldown is in progress.*



*Figure 19-3. The storage dewar in the foreground is supplying liquid nitrogen to the neuro cooldown dewar in the background.*

Immediately after completion of cryoprotective perfusion, when crackphone sensors and thermocouples have been placed, a steel screw-eye is inserted into the severed base of the patient's spine, below the head. This allows the cephalon to be handled with minimal risk of damage. It is lowered by the screw-eye into a small LR40 dewar, and the mouth of the dewar is filled with a styrofoam plug about five inches thick. Nitrogen is supplied from a small reservoir dewar (seen in the foreground in Figure 19-3).

Initial cooling is a rapid plunge to  $-110$  degrees, a temperature which Hixon chose because it is comfortably higher than  $-117$  degrees, which is the highest temperature at which he has ever observed a fracturing event. (If a patient has been perfused with glycerol as opposed to M22 cryoprotectant, the rapid plunge terminates at  $-85$  degrees.)

After the plunge, the gradual cooling phase begins. This is controlled by software which uses an initial temperature, a final temperature, and the desired cooling period to calculate a gradient which is approximately linear.

The software controls a solenoid valve that allows short bursts of liquid nitrogen from the reservoir dewar to pass into the neuro dewar. Each burst lasts about 0.7 second, and the bursts are separated by intervals of up to several minutes. Temperature is monitored using thermocouples that were placed in the cephalon, in addition to thermocouples measuring the vapor temperature inside the neuro dewar. Slow cooling from  $-110$  degrees to liquid-nitrogen temperature takes about three days.

Typically, crackphone monitoring detects 15 to 25 fracturing events during this process. Hixon has tried changing the cooldown rate from 1 degree per hour to 0.25 degrees per hour, but feels that the number of events was about the same.

When cooldown is complete, the neuro dewar is slowly filled with liquid nitrogen. This causes an abrupt reduction in temperature of about 2 degrees. A laser is used to detect the rising liquid level.

A cube-shaped styrofoam box with detachable lid is prepared for neuro transfer. A cylindrical aluminum storage cylinder measuring approximately 10 inches in diameter and 12 inches in height is placed in this box. An example is shown in Figure 19-4. This cylinder, commonly referred to as a “neuro cannister,” is lined internally with soft Dacron fiber. The fiber is saturated with liquid nitrogen, and a small pool of liquid nitrogen is poured into the bottom of the can, before the cephalon is lowered into it. The can is then filled with liquid nitrogen, and an aluminum lid is wired into place. The lid and the can are labeled with the date and the patient identification number. The can is moved to a storage dewar for immersion in liquid nitrogen.



Figure 19-4. A cannister for long-term maintenance of a neuro patient at Alcor Foundation.

## **Neuropatient Cooldown at Cryonics Institute**

Prior to the arrival of Ben Best, the Cryonics Institute did not offer neuropreservation as an option. When Best took over as president of the organization, he designed a box-shaped insulated enclosure for cooling cephalons using liquid nitrogen vapor circulated by an internal fan under computer control. This is a smaller-scale version of the whole-body cooling system at CI, described above.

## **Cooldown Following Field Neuro-Vitrification**

The initial rapid cooling of a cephalon following field vitrification is achieved simply by surrounding the cephalon with dry ice in the transport box. This system has been discussed in Section 16 discussing remote blood washout.

## **Whole-Body Cooldown Box at Suspended Animation**

During 2005, staff at Suspended Animation fabricated a proof-of-concept cooldown box at the Boynton Beach facility in Florida. This is shown in Figure 19-5.





*Figure 19-5. Prototype rapid cooldown box designed by Charles Platt and Gary Battiato at Suspended Animation in 2005. The fans were driven by motors mounted externally on the lid of the box. Sheets of foam board were used as insulation.*

Plywood was used for speed and economy of manufacture, but if the system had been fully developed, it would have been rebuilt from stainless steel and/or aluminum.

Liquid nitrogen was injected via a perforated galvanized pipe visible around three edges of the interior. The patient would be introduced to the box on a stainless-steel tray (visible at bottom-right), through a hinged door at the

end of the box. Fans circulated vapor through a hollow lid, which could be opened, as in the photograph, to allow maintenance access.

The box was tested using bags filled with liquid to simulate the mass of a patient. After two trials, development was discontinued. More detailed analysis suggested that the vapor circulation rate may have been unnecessarily high in this prototype.

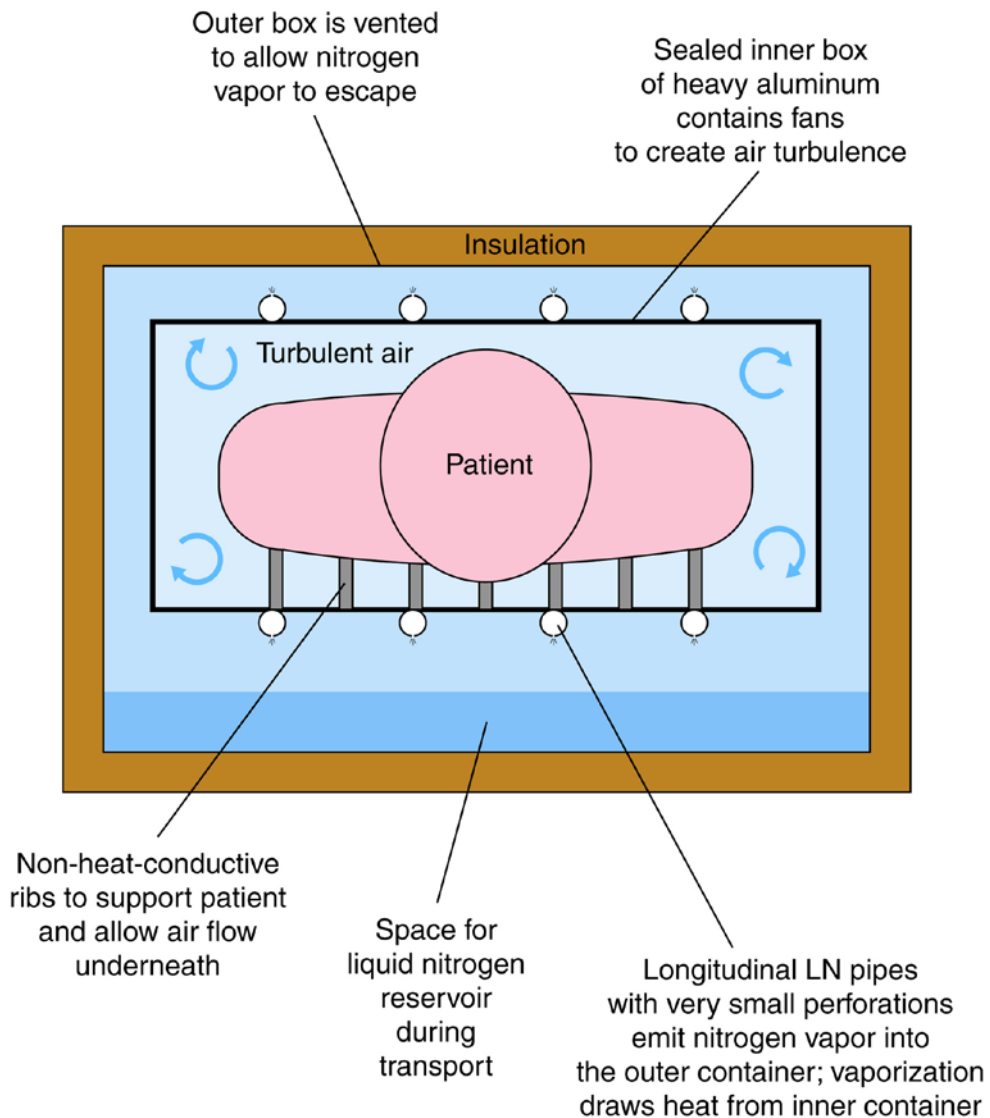
## **Other Alternatives**

In 2008 Suspended Animation was asked to develop a whole-body rapid cooldown enclosure that would increase the cooling rate while minimizing localized temperature variations. Hypothetically, the enclosure might be used for cooldown during transport in a specially designed ground vehicle after remote whole-body washout.

The change of state at the moment when liquid nitrogen boils will absorb far more heat than cold vapor after boiling has occurred. Therefore, the SA design promoted boiling inside a network of tubing, so that the tubes would absorb heat.

Preliminary sketches suggesting this method are shown in Figure 19-6 (end view) and Figure 19-7 (side view). Inside an enclosure, air circulates around the patient to eliminate “hot spots.” Around the enclosure, liquid nitrogen is introduced through tubing, and either vaporizes inside the tubing and is allowed to escape through small perforations. The tubing absorbs heat from the interior shell of the box.





*Figure 19-6. Sketch for an enclosure to provide a controlled temperature environment for cooling with maximum efficiency and uniformity, in a hypothetical scenario for patient transport after remote whole-body blood washout.*

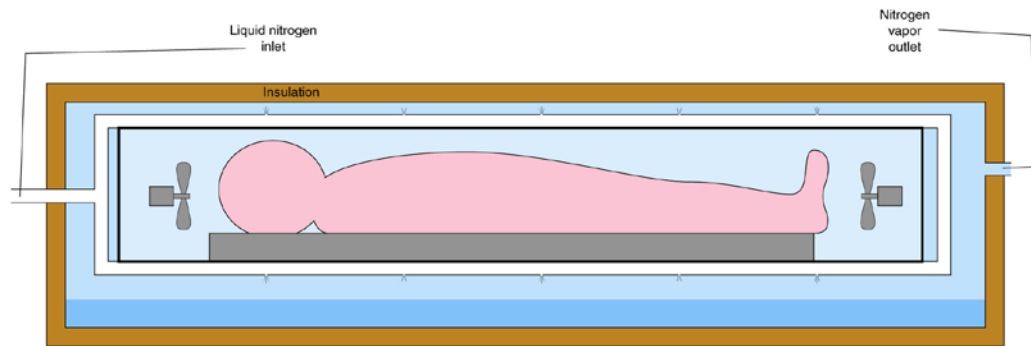


Figure 19-7. Side view of the cooling enclosure shown in Figure 19-6.

Suspended Animation also created a 3D rendering of a proof-of-concept version. It proposed a hollow framework of one-inch square-section aluminum tubes, as shown in Figure 19-8. All of the tubes would be open to each other internally, by drilling holes in them before welding them together. Liquid would be introduced in the tube at the bottom, and would rise up the side tubes, vaporizing as it did so. It would be vented from a short vertical tube at the far end.



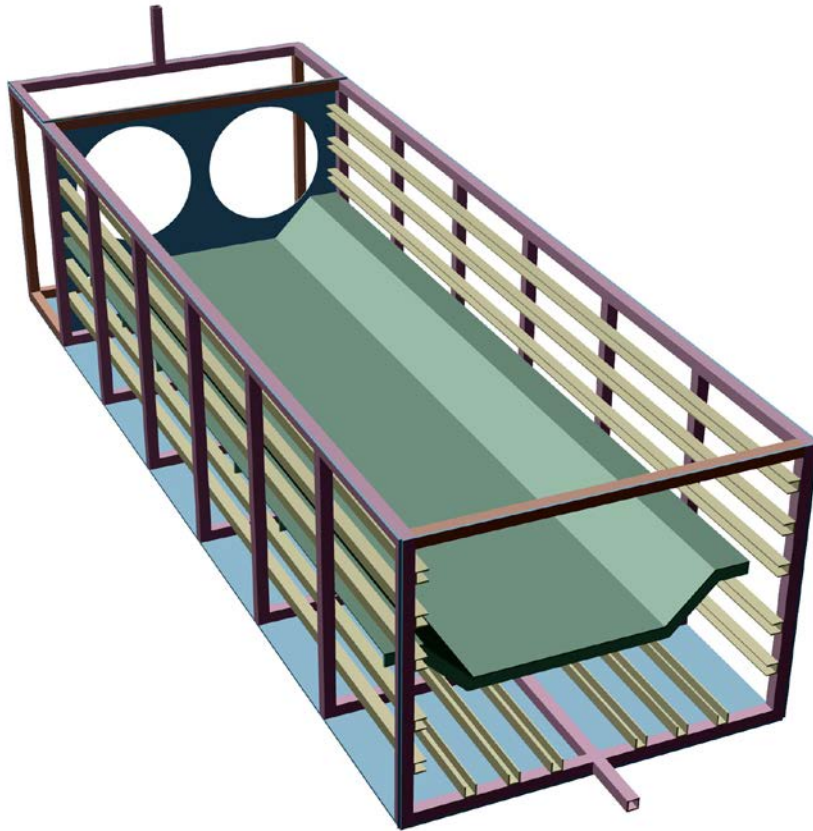
*Figure 19-8. 3D rendering of a design for rapid cooling. The square-section tubing is hollow and is internally interconnected. Liquid nitrogen is injected into the tube at the bottom, and vapor is vented through the short vertical tube at the far end.*

In addition, channel-section aluminum strips would be mounted inside the box, to act as a giant heat sink, as shown in Figure 19-9. Ultimately a tray could be inserted to support the patient, as shown in Figure 19-10. Two fans at the far end would draw air over the patient and recirculate it under the tray.

A master welder who was employed by Suspended Animation at that time constructed the skeletal form of the cooling system shown in Figure 19-9, excluding the fan panel at the end. Sheets of 16-gauge aluminum were added around the framework, and foamboard thermal insulation was attached to all six exterior faces of the box.



*Figure 19-9. The design from Figure 19-8 with channel-section aluminum strips added to act as heat sinks. Color has been added to enable easier identification of the parts, and has no other significance.*



*Figure 19-10. The design from Figure 19-9 with a tray added to support a patient. Fans would be used in the panel at the far end, to circulate air internally. (A tray was never added to the prototype that was built.)*

An initial test was performed to find out if vapor locks would occur. This was a significant concern, because vapor might form under liquid in some of the tubes with unknown consequences.

Liquid nitrogen was introduced, and vapor from the end tube was piped through a two-inch hose that exhausted outside the facility. After approximately one hour, the interior of the box had stabilized at approximately  $-160$  degrees Celsius. No vapor locks occurred, and the experiment ended.

When the box was broken down for inspection, small cracks were found where side ribs had been welded to the central tube at the bottom. An engineer with experience in high-temperature steam tubing believed that the cracks had

been created by thermal contraction of the bottom tube, and suggested that the problem could be eliminated by using standard practice whereby direct runs of tubing should be eliminated in favor of a zig-zag pattern that would distribute contraction forces.

Unfortunately the welder was unable to prolong his stay at the design facility in California, and these modifications were never made. At the time of writing, any plans for rapid-cooling equipment at Suspended Animation do not appear to exist.

## **Developments at Alcor**

When Tanya Jones and Steve van Sickle acquired administrative roles at Alcor Foundation in 2005, they announced that they were going to re-think and re-engineer equipment used in all phases of cryonics cases. All of the standby kits that had been deployed around the country would become obsolete, a new ice bath would be designed, a new field-perfusion system would replace the Air Transportable Perfusion kit (ATP), and a new system enabling whole-body cooldown would be installed at Alcor's facility. This would be a dual-purpose enclosure so that perfusion and rapid cooldown could occur within the same equipment..

At an open house where Alcor displayed some of the new designs, van Sickle mentioned that the perfusion-and-cooldown enclosure had been tested to -79 degrees Celsius, but a few years later, Hixon stated that the design had suffered from heat losses to such an extent that it wasn't practical. Some modifications were made, but the prototype experienced other problems and no longer exists. While a single enclosure for perfusion and cooldown may still be an attractive concept, there are no plans to pursue this at Alcor at the time of writing.

## **Developments at Cryonics Institute**

Following the departure of Ben Best, we are not aware of subsequent cooldown innovations proposed or implemented at CI.